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A Solid-Supported, Enantioselective Synthesis Suitable for the Rapid Preparation of Large Numbers of Diverse Structural Analogues of (−)-Saframycin A

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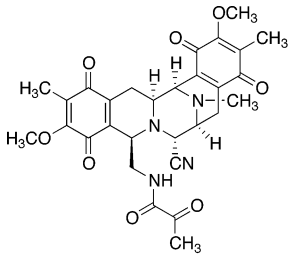
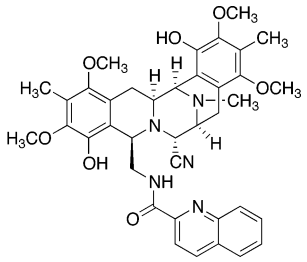
Saframycin A (**1**) is a structurally complex alkaloid that is a member of a series of natural antiproliferative agents containing a cyanopiperazine core, or its functional equivalent, within a complex polycyclic framework.[1] Members of this series have shown promising clinical efficacy in the treatment of solid tumors and have proven amenable to structural modification in the search for analogues with improved pharmacological properties.[2,3] Typically, analogue synthesis has been restricted to the derivatization of the natural products or to the late-stage modification of advanced intermediates prepared by multistep solution-phase synthesis. In one such study, we recently showed that a series of bis-hydroquinone dimethyl ether derivatives of saframycin A (see structure **2**) displayed enhanced antiproliferative activity in two cancer cell lines.[3] Evidence from transcriptional-profiling experiments conducted with a susceptible yeast strain suggested that **1** and **2** function by a common mechanism despite their considerable structural differences.[4]



(–)-Saframycin A (1)



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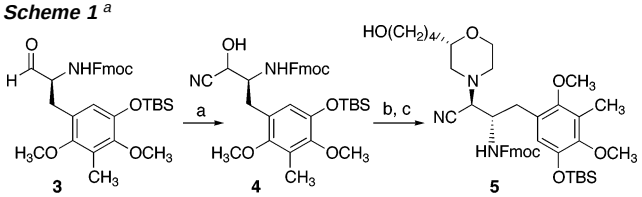
Here, we describe the successful adaptation of our prior solution-phase synthesis of saframycin A[5] to a 10-step solid-supported synthesis suitable for the preparation of large numbers of diverse saframycin analogues with deep-seated structural modifications. We illustrate the utility of this route by constructing a matrix of 16 saframycin analogues through simultaneous parallel synthesis employing two points of structural variability (Table 2). This work is notable not only as a preliminary step toward large-scale library construction, but also as an example of the use of sequential stereoselective C−C bond forming reactions on the solid phase for the preparation of natural product analogues.[6]

Our prior solution-phase route to saframycin A (**1**) involved the directed condensation of α-amino aldehyde components with protective groups masking either the aldehyde or amino functional groups (C- or N-protected, respectively). In analogy to the solid-phase synthesis of peptides,[7] we envisioned an adaptation of this solution-phase synthesis to the solid phase by using the resin support to covalently tether the "C-terminal" α-amino aldehyde component that initiated the condensation sequence. Assembly of the saframycin skeleton would then occur by a short series of condensation reactions proceeding with C → N directionality. The transition to the solid phase required careful optimization of the resin, the linker, and the conditions for each step of the 10-step sequence.

In the successful route, a novel dual linker[8] was developed for resin immobilization of the first α-amino aldehyde component (compound **3**, Scheme 1).[9] This linker took the form of the

Scheme 1 [a]



[a] Reaction conditions: (a) KCN, AcOH, CH_3OH, CH_2Cl_2, 23 °C, 87%. (b) (*S*)-2-[4-(*tert*-butyldimethylsiloxy)-1-butyl]morpholine, CF_3CH_2OH, −20→23 °C, 83% (syn:anti 1.2:1). (c) 1% HCl−CH_3OH, 0 °C, 94%.

secondary amino alcohol derivative (*S*)-2-[4-(*tert*-butyldimethylsiloxy)-1-butyl]morpholine, prepared in large amounts by a simple seven-step sequence featuring the Jacobsen hydrolytic kinetic resolution (HKR, see Supporting Information).[10] The dual linker was attached to the C-terminus of **3** by amino nitrile formation under previously optimized conditions,[11] forming a mixture of syn and anti diastereomers (dr 1.2:1, respectively, anti stereochemistry depicted, Scheme 1). Amino nitrile formation occurred without significant epimerization of the α-stereocenter (<2%), as anticipated. To simplify the spectroscopic analysis of intermediates produced subsequently in the synthetic route, the syn and anti diastereomers were separated, and the route was optimized using the pure anti isomer (**5**). This proved to be a fortuitous decision, for the syn diastereomer was later found not to transform with the same efficiency in the synthetic route, an unexpected result given that (2-unsubstituted) *syn*- and *anti*-morpholino nitriles were essentially interchangeable in the prior solution-phase route.[12]

Attachment of the *anti*-morpholino nitrile **5** to the solid support was achieved by silyl ether formation with 4-(chlorodiisopropylsilyl)polystyrene,[13] providing the first resin-bound intermediate (**6**, Scheme 2) in quantitative yield.[14] Selective deprotection of the *tert*-butyldimethylsilyl ether group of resin-bound intermediate **6** occurred upon exposure to tetrabutylammonium fluoride buffered with acetic acid; subsequent treatment with piperidine in DMF unmasked the amino terminus, affording the phenolic amine **7**. Addition of a 3-fold excess of the N-protected α-amino aldehyde **3** to the amino-terminal intermediate **7** provided the corresponding resin-supported imine, which was thoroughly washed to remove excess **3**, as well as water produced during the condensation reaction. Warming the imine intermediate at 35 °C in a saturated solution of anhydrous lithium bromide in 1,2-dimethoxyethane

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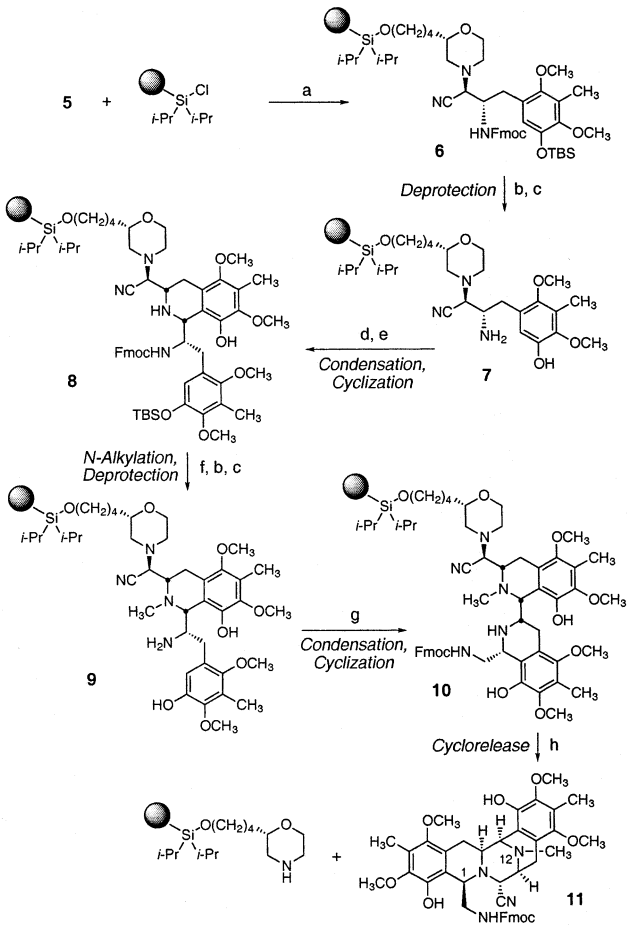
Scheme 2 [a]



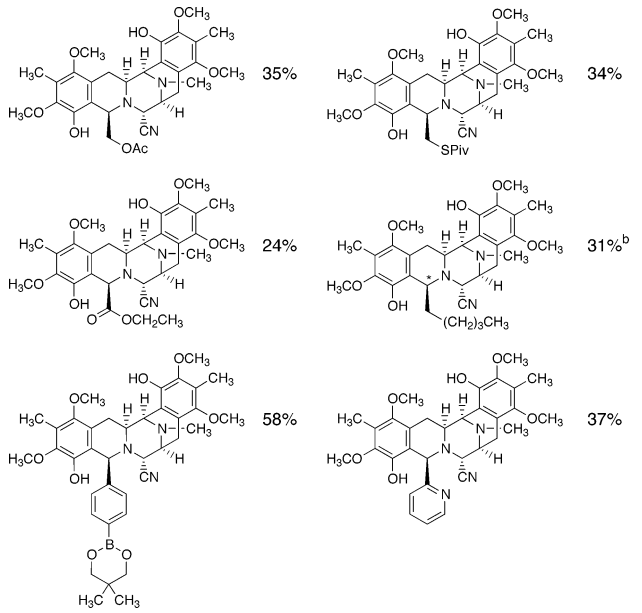
[a] Reaction conditions: (a) Imidazole, DMF, 23 °C; CH_3OH, imidazole, 23 °C, 99%. (b) TBAF, AcOH, THF, 23 °C. (c) Piperidine, DMF, 23 °C. (d) **3** (3.0 equiv), DMF, 23 °C. (e) LiBr, DME, 35 °C, 81% (4 steps). (f) CH_2O-H_2O, $NaBH(OAc)_3$, DMF, 23 °C, 95%. (g) *N*-Fmoc glycinal, DCE, 40 °C, 100% (3 steps). (h) $ZnCl_2$, 4 Å molecular sieves, THF, 55 °C, 70%. Resin support is polystyrene (2% DVB), 150−300 μm.

induced a stereoselective Pictet−Spengler cyclization reaction, affording the *cis*-tetrahydroisoquinoline derivative **8** (cis:trans 7:1). The four-step sequence from **6** to **8** proceeded in 81% yield. The use of the particular combination of dual linker and solid support described proved crucial to the success of the Pictet−Spengler cyclization; solid supports such as Tentagel could not be dried sufficiently to preclude hydrolysis of the imine intermediate, and alternative linkers gave rise to inefficient or nonstereoselective Pictet−Spengler cyclization reactions.

The secondary amino group of the tetrahydroisoquinoline intermediate **8** formed in the first Pictet−Spengler cyclization was next reductively methylated on the solid phase. Subsequent deprotection of the phenol and primary amino groups of the resulting N-alkylation product produced the new amino-terminal resin-bound intermediate **9**. The second and final Pictet−Spengler cyclization reaction in the sequence then occurred (concomitantly with imine formation) upon exposure of **9** to *N*-Fmoc glycinal (3 equiv) in 1,2-dichloroethane at 40 °C for 20 h. The resulting bis-tetrahydroisoquinoline derivative **10** was formed in quantitative yield with the required cis stereochemistry in the newly formed ring.

In the key step of the solid-phase sequence, the bis-tetrahydroisoquinoline intermediate **10** was subjected to cyclization−autorelease[15] by warming in the presence of zinc chloride (3 equiv) at 55 °C for 1.5 h. This transformation is presumed to involve

Table 1. Bis-hydroquinone Analogues Prepared by Variation of the Aldehyde Substrate in the Final Pictet−Spengler Cyclization of a 10-Step Solid-Supported Sequence[a]



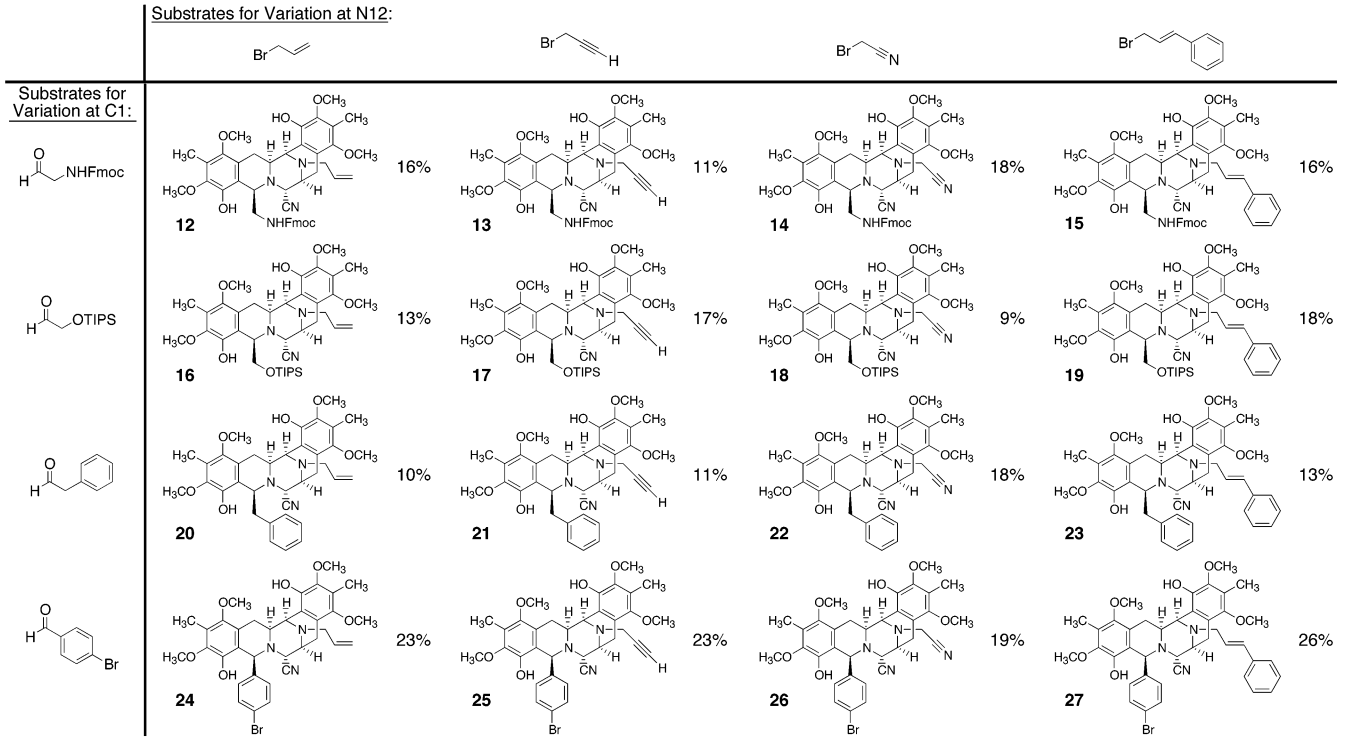
[a] Yields over 10 steps (from **5**). [b] Obtained as a 1.4:1 mixture of cis:trans isomers; in every other case, the cis:trans ratio exceeded 20:1.

reversible morpholinium ion formation through expulsion of cyanide, internal capture by cyclization of the secondary amino group (formed in the final Pictet−Spengler cyclization), and subsequent extrusion of the resin-bound morpholine dual linker through its secondary point of attachment (the amino group). The newly liberated iminium ion intermediate is presumably then captured in solution by cyanide, providing the saframycin analogue **11** directly, in a remarkable yield of 53% for the 10-step sequence from **5**. A key feature of this cyclization−autorelease strategy is its diastereospecificity. Only the diastereomer shown is capable of cyclization; thus, minor diastereomeric impurities that are formed during the synthesis (e.g., **7** → trans-**8**) remain on the resin. This likely accounts, in part, for the high purity of the product obtained (**11**, >95% purity, ^{1}H NMR analysis).

With the successful development of a solid-phase synthesis of the saframycin A analogue (and direct precursor[16]) **11**, we sought to evaluate the versatility of the sequence toward structural modification by variation of the aldehydic reactant in the final Pictet−Spengler cyclization of the route (see **9** → **10**). Heating intermediate **9** with a wide range of aliphatic and aromatic aldehydes (3−5 equiv) followed by cyclorelease, as before, provided a series of saframycin analogues in 24−58% yield over the 10-step sequence (Table 1).

To explore a second site for structural diversification, we examined alternative N-alkylation reactions of the secondary amino group of the solid-supported tetrahydroisoquinoline intermediate **8**. Alkylation of **8** with a range of commercially available alkyl bromides occurred readily in DMF containing anhydrous lithium iodide and *N*,*N*-diethylaniline as base (illustrated within Table 2, see below). We next sought to explore the feasibility of simultaneous variation of the two sites of structural diversification identified. By employing a range of alkyl bromides in the N-alkylation of resin **8** and simultaneously varying the aldehydic reactant in the second Pictet−Spengler cyclization, a 16-membered library of saframycin A analogues bearing varied C1 and N12 substituents was prepared by parallel synthesis in an array of half-

Table 2. Simultaneous Parallel Synthesis of Saframycin Analogues in a 10-Step Solid-Supported Sequence[a]



[a] Yields over 10 steps from **5**.

dram glass vials (Table 2); a uniform set of reaction conditions was employed throughout, in all cases providing saframycin analogues as single compounds following cyclorelease. The cleaved analogues were isolated by simple filtration through silica gel plugs (to remove zinc chloride) and concentration. The synthesis provided 0.5−2.0-mg quantities of the bis-hydroquinone products in 9−26% overall yield (from **5**), typically in ∼95% purity,[17] representing an average yield of 79−87% per step over the 10-step solid-phase sequence.

In summary, we describe the solid-supported synthesis of 23 saframycin analogues, including 16 prepared by simultaneous parallel synthesis. The route, which bears analogy to solid-phase peptide synthesis, involved the directed condensation of N-protected α-amino aldehyde reactants using a novel dual linker both for attachment to the solid support and in a diastereospecific cyclorelease mechanism. By supporting structural variation at multiple sites in the saframycin core while simultaneously obviating the need for chromatographic purification of library members, this synthesis should be suitable for the production of libraries of (conservatively) hundreds of structurally diverse saframycin analogues.

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Supporting Information Available: Experimental procedures for the preparation of bis-hydroquinone **11** and tabulated spectroscopic data for all synthetic analogues (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reviews: (a) Arai, T.; Kubo, A. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1983; Vol. 21, Chapter 3. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley-Interscience: New York, 1988; Vol. 2, Chapter 3.

(2) (a) Martinez, E. J.; Owa, T.; Schreiber, S. L.; Corey, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3496−3501. (b) Danishefsky, S. J.; Zhou, B. U.S. Pat. Appl. 20020025962, Feb 28, 2002.

(3) Myers, A. G.; Plowright, A. T. *J. Am. Chem. Soc.* **2001**, *123*, 5114−5115.

(4) (a) Plowright, A. T.; Schaus, S. E.; Myers, A. G. *Chem. Biol.* **2002**, *9*, 607−618. Transcriptional profiles of Et-743 and the more potent synthetic analogue phthalascidin in two human cancer cell lines were likewise found to be nearly identical: (b) Martinez, E. J.; Corey, E. J.; Owa, T. *Chem. Biol.* **2001**, *8*, 1151−1160.

(5) Myers, A. G.; Kung, D. W. *J. Am. Chem. Soc.* **1999**, *121*, 10828−10829.

(6) Review: Hall, D. G.; Manku, S.; Wang, F. *J. Comb. Chem.* **2001**, *3*, 125−150.

(7) Reviews: (a) *Solid-Phase Peptide Synthesis*, Fields, G. B., Ed.; Academic Press: San Diego, 1997. (b) *Fmoc Solid-Phase Peptide Synthesis: A Practical Approach*, Chan, W. C.; White, P. D., Eds.; Oxford University Press: New York, 2000.

(8) (a) Tam, J. P.; Tjoeng, F. S.; Merrifield, R. B. *Tetrahedron Lett.* **1979**, *20*, 4935−4938. (b) Tam, J. P.; Tjoeng, F. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 6117−6127.

(9) Myers, A. G.; Kung, D. W.; Zhong, B.; Movassaghi, M.; Kwon, S. *J. Am. Chem. Soc.* **1999**, *121*, 8401−8402.

(10) Tokunaga, M.; Larrow, J. F.; Kakiuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936−938.

(11) Myers, A. G.; Zhong, B.; Kung, D. W.; Movassaghi, M.; Lanman, B. A.; Kwon, S. *Org. Lett.* **2000**, *2*, 3337−3340.

(12) This is presumably a consequence of the introduction of the "remote" stereocenter at C-2 of the morpholine nucleus, rather than an idiosyncrasy of solid- versus solution-phase chemistry, but this has not yet been established experimentally.

(13) Randolph, J. T.; McClure, K. F.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 5712−5719.

(14) Unreacted silyl chloride sites on the resin were capped with methanol. Yields in solid-phase transformations were determined spectrophotometrically from the UV absorption of dibenzofulvene released upon treatment with 20% piperidine−DMF and were confirmed in several cases by product isolation following acid-catalyzed cleavage of the silyl ether-linked intermediates.

(15) Reviews: (a) Van Maarseveen, Jan. H. *Comb. Chem. High Throughput Screening* **1998**, *1*, 185−214. (b) Seitz, O. *Nachr. Chem.* **2001**, *49*, 912−916.

(16) We have previously shown that the pentacyclic intermediate **11** can be converted into saframycin A in 52% yield (3 steps, see ref 5).

(17) As determined by ^{1}H NMR analysis, with two exceptions: products **20** and **21** were ∼80% pure, containing impurities from self-condensation of phenylacetaldehyde.

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